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Development of a physiology-directed population pharmacokinetic and pharmacodynamic model for characterizing the impact of genetic and demographic factors on clopidogrel response in healthy adults

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ABSTRACT

Clopidogrel (Plavix®), is a widely used antiplatelet agent, which shows high inter-individual variability in treatment response in patients following the standard dosing regimen. In this study, a physiology-directed population pharmacokinetic/pharmacodynamic (PK/PD) model was developed based on clopidogrel and clopidogrel active metabolite (clop-AM) data from the PAPI and the PGXB2B studies using a step-wise approach in NONMEM (version 7.2). The developed model characterized the *in vivo* disposition of clopidogrel, its bioactivation into clop-AM in the liver and subsequent platelet aggregation inhibition in the systemic circulation reasonably well. It further allowed the identification of covariates that significantly impact clopidogrel's dose–concentration–response relationship. In particular, CYP2C19 intermediate and poor metabolizers converted 26.2% and 39.5% less clopidogrel to clop-AM, respectively, compared to extensive metabolizers. In addition, CES1 G143E mutation carriers have a reduced CES1 activity (82.9%) compared to wild-type subjects, which results in a significant increase in clop-AM formation. An increase in BMI was found to significantly decrease clopidogrel's bioactivation, whereas increased age was associated with increased platelet reactivity. Our PK/PD model analysis suggests that, in order to optimize clopidogrel dosing on a patient-by-patient basis, all of these factors have to be considered simultaneously, e.g. by using quantitative clinical pharmacology tools.

1. Introduction

Clopidogrel (Plavix®), a second generation thienopyridine platelet inhibitor, has been the standard-of-care for treating patients with acute coronary syndrome (ACS) and/or undergoing percutaneous coronary interventions (PCI) in recent years (Wright et al., 2011). Although clopidogrel is generally considered safe and effective, considerable variability in treatment response has been reported for many patients (Perry and Shuldiner, 2013). It is thought that approximately 20–44% of patients receiving the standard clopidogrel dosing regimen (300 mg loading dose, 75 mg maintenance dose) do not receive the full treatment benefit, which results in high on-treatment platelet reactivity (HPR) and continued cardiovascular events (Angiolillo et al., 2007; Geisler et al., 2008; Gurbel et al., 2003; Gurbel and Tantry, 2007; Matetzky et al., 2004; Taubert et al., 2006). On the other hand, some patients also experience drug-induced bleeding due to excessive platelet inhibition (Yusuf et al., 2001).

Clopidogrel itself is an inactive pro-drug that requires enzymatic conversion into its active metabolite (clop-AM) by a number of cytochrome P450 (CYP) enzymes (Kazui et al., 2010; Patrono, 2009). Once formed, clop-AM binds irreversibly to the P2Y12 receptor, which is located on the surface of platelets, where it inhibits platelet aggregation and decreases platelet reactivity for the platelet's life span. Following oral administration, about 50% of the clopidogrel dose is absorbed from the gut prior to entering the liver (Sanofi-Aventis, 2011), where the drug undergoes extensive first-pass metabolism. Most of the drug (85–90%) is hydrolyzed by liver-specific carboxylesterase 1 (CES1) to the inactive carboxylic acid metabolite SR26334 (Bonello et al., 2010), while the remainder is converted to the active metabolite R-130964 (clop-AM) in a two-step bioactivation process (Kazui et al., 2010). It should be noted that in addition to the breakdown of clopidogrel, CES1 is also involved in the metabolism of clopidogrel's intermediate and active metabolites in the liver (Bouman et al., 2011; Zhu et al., 2013). As a result, only about 2% of the administered clopidogrel dose reaches the systemic circulation where it becomes available for exerting its pharmacological effect (Sanofi-Aventis, 2011).

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In vitro enzyme kinetic data using cDNA-expressed human P450 isoforms suggests that CYP1A2 (35.8%), CYP2B6 (19.4%) and CYP2C19 (44.9%) contribute to the formation of 2-oxo-clopidogrel, whereas CYP2B6 (32.9%), CYP2C9 (6.79%), CYP2C19 (20.6%) and CYP3A4 (39.8%) contribute to the formation of clop-AM, respectively (Kazui et al., 2010), suggesting that CYP2C19 is responsible for about 50% of the overall clop-AM formation. Polymorphisms in the gene encoding for CYP2C19 will consequently have a significant impact on clopidogrel's dose–concentration–response relationship. This is in line with previous literature reports, where CYP2C19 was identified as one of the most important polymorphic CYP enzymes across different populations. Approximately 3–5% of Caucasians and 15–20% of Asians are CYP2C19 poor metabolizers (PMs) with no remaining CYP2C19 functionality (Desta et al., 2002). The importance of CYP2C19 on clopidogrel treatment effect has been confirmed by numerous clinical studies, which show that patients with decreased CYP2C19 activity, i.e. intermediate metabolizers (IMs) or PMs, have remarkably higher on-treatment platelet reactivity and, thus, an increased risk in experiencing ischemic events following administration of the standard dosing regimen. As a consequence, FDA issued a boxed warning for patients with decreased CYP2C19 activity (FDA, 2010; Hochholzer et al., 2010; Shuldiner et al., 2009; Siller-Matula et al., 2012; Simon et al., 2009). Several clinical studies also revealed that, in addition to CYP2C19 polymorphism, multiple other demographic and disease-related risk factors contribute to the between-subject variability in clopidogrel treatment response (Frelinger et al., 2013; Hulot et al., 2006; Mega et al., 2009; Siller-Matula et al., 2012; Simon et al., 2009). For example, age was identified as covariate in older ACS patients, who have significantly higher on-treatment platelet reactivity than their younger peers (Cuisset et al., 2011; Hochholzer et al., 2010; Shuldiner et al., 2009). It has also been shown that obesity significantly affects the response to clopidogrel treatment as a result of lower systemic exposure to clop-AM and, thus, reduced platelet inhibition (Shuldiner et al., 2009; Wagner et al., 2013). Diabetes mellitus leads to significantly higher on-treatment platelet reactivity (Hochholzer et al., 2010) at least partially due to the suppressions of CYP2C19 activity and, thus, lower systemic clop-AM exposure (Bogman et al., 2010; Erlinge et al., 2008). Findings from recent *in vitro* studies further suggest that changes in CES1 activity also greatly impact clopidogrel's dose–concentration–response relationship (Zhu et al., 2013). These findings were confirmed in healthy adults and patients undergoing PCI, who were carriers of CES1 loss-of-function alleles (Lewis et al., 2013b). In order to appropriately account for inter-individual differences in response to clopidogrel treatment, all of these factors as well as their dynamic interplay have to be accounted for simultaneously, which is difficult if not impossible to achieve in head-to-head clinical trial settings. This challenge may be met by the use of modeling and simulation approaches as they allow for the integration of data from different *in vitro*, animal and clinical data into a single unifying model (Jiang et al., 2015). Once established and qualified, this model can then be used to explore clinically yet unstudied scenarios or combinations of different covariates and thus guide dose selection as well as the design of future clinical trials.

The objective of this study was to develop a physiology-directed population pharmacokinetic pharmacodynamic (pop-PK/PD) modeling and simulation framework using data from the Pharmacogenomics of Antiplatelet Intervention (PAPI) and PGXB2B studies that allows to simultaneously evaluate the impact of multiple demographic and genetic factors on clopidogrel's dose–concentration–response relationship on a physiological rather than a purely descriptive basis. The proposed framework expands on conventional PK/PD approaches as it specifically takes information on the biotransformation site as well as respective physiological parameters into consideration. Its mechanistic nature uniquely positions the developed framework as knowledge platform that can be readily expanded to evaluate the impact of newly identified polymorphisms or covariates to determine their overall impact on platelet reactivity and ultimately to guide dose selection for an individual patient.

2. Methods

2.1. Clinical studies

2.1.1. Amish PAPI study

Study details, recruitment and population characteristics of the Amish PAPI study (NCT0079936) are described elsewhere (Lewis et al., 2013a; Shuldiner et al., 2009). The current analysis utilized an expanded set of 605 healthy male and female Amish Caucasian individuals recruited from August 2006 to January 2011 (Table 1). Briefly, the use of all medications, vitamins and supplements were discontinued 1 week before the initiation of the study. Information on all participants' medical and family history was obtained. Physical examinations, blood samples, anthropometric measures and extensive phenotypic measurements were conducted after an overnight fast. Following baseline platelet aggregation measurement, all participants were given a 300 mg loading dose of clopidogrel followed by 75 mg maintenance dose per day for 6 days. At one hour following the last clopidogrel dose, a blood sample was drawn, platelet aggregation, clopidogrel and clop-AM concentrations were obtained.

2.1.2. PGXB2B study

Healthy Amish subjects who had participated previously in the PAPI (NCT0079936) study were recruited in the PGXB2B study (NCT01341600). The study details and population characteristics were previously reported (Horenstein et al., 2014). Briefly, of the 18 healthy male and female adults, 6 were CYP2C19 extensive metabolizers (EMs, *1/*1 and *1/*17), 6 were CYP2C19 IMs (*1/*2 and *2/*17), and 6 were CYP2C19 PMs (*2/*2), as shown in Table 1. The use of all medications, vitamins and supplements was discontinued 1 week before the initiation of the study. Information on all participants' medical and family history was obtained. Physical examinations, blood samples, anthropometric measures and extensive phenotypic measurements were conducted after an overnight fast. All subjects received 75, 150 or 300 mg clopidogrel once daily for 8 days in a crossover study design. Plasma concentrations of clopidogrel and clop-AM were measured at 0.25, 0.5, 1, 2 and 4 h on day 1 after drug administration at each dose. Platelet reactivity was determined at baseline as well as 4 h after drug administration on day 1 and day 8 at each dose. Drug was washed out for one week after each dose treatment.

2.2. Genotyping

Genotyping methods have already been reported in detail (Horenstein et al., 2014; Lewis et al., 2011, 2013a, 2013b; Shuldiner

Table 1
Demographic and pharmacogenetic characteristics of PAPI (N = 605) and PGXB2B (N = 18) subjects assessed in the population pharmacokinetic/pharmacodynamic analysis.

Baseline characteristics	PGXB2B (N = 18)	PAPI (model estimation) (N = 480)	PAPI (model qualification) (N = 125)
Age, y	43 ± 10	44 ± 13	44 ± 14
Male	11	237	64
Weight, kg	79.8 ± 12.9	75.3 ± 12.7	74.9 ± 13.8
Height, cm	169 ± 8	167 ± 8	167 ± 9
BMI, kg/m^2	28.1 ± 4.7	27.1 ± 4.6	26.8 ± 4.9
CYP2C19*1/*1	4	145	45
CYP2C19*1/*2	5	103	24
CYP2C19*2/*2	6	7	7
CYP2C19 *1/*17	2	147	31
CYP2C19 *2/*17	1	54	5
CYP2C19 *17/*17	0	24	13
CES1 CC	17	473	125
CES1 TC	1	7	0
PON1 AA	8	223	61
PON1 AG	8	201	52
PON1 GG	2	56	12